



Fluorescent biosensor for hyaluronidase

intensity based ratiometric sensing and time-gated detection using a long lifetime azadioxatriangulenium (ADOTA) fluorophore

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side, which is most likely related to different base pairing at the two miRNA-155 termini. The highest translocation rates were obtained with the dual extended DNA probe but the shortest dwell times with the 3'-only extension. These optimized parameters enable nanopore resistive pulse sensing of miRNA molecules over a larger concentration range.

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Nanopore as a Sensor Based on Avidin-Biotin System

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This work aims to design a highly selective biosensor, able to detect selectively a molecule with a label or with an antigen/antibody system, and without the use of a microscope or a laser, and thus building a low cost detection system.

To make this we have explored different strategies used to fashion artificial nanopore in order to mimic biological ligand-gated channel. This strategy is based on PEG like-avidin tethering in track etched nanopore modified by atomic layer deposition (ALD). Track etched combined with ALD was chosen to control their geometry and the nanopore diameter (cylindrical) and to functionalize them with polymers and biomolecules.

Then we have investigated the sensing aspect more in details. (i) the size of the spacer, with a long or short PEG chain, (ii) compare avidin and streptavidin, (iii) biotinylated proteins: IgG and BSA, (iv) and the addition of an antibody (anti BSA).

This new strategy to tailor artificial ligand-gated nanopore permits to consider a simple and low cost biosensor for antibody detection.

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Fluorescent Biosensor for Hyaluronidase: Intensity Based Ratiometric Sensing and Time-Gated Detection using a Long Lifetime Azadioxatriangulenium (ADOTA) Fluorophore

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We have designed a rapid and sensitive, intensity based ratiometric sensing and lifetime based sensing probe for the detection of hyaluronidase activity. Hyaluronidase has been reported to have upregulated in various pathological conditions like cancer. Therefore, developing an assay to estimate the enzyme concentration is of utmost importance and can be used as a diagnostic method for cancer. We have developed a fluorescent probe by using hyaluronic acid as template and heavily labeling it with an orange/red emitting azadioxatriangulenium (ADOTA) fluorophore, which also has a long fluorescence lifetime. The heavily ADOTA labeled hyaluronic acid (HA-ADOTA) has a red shift in the peak emission wavelength (605 nm), a weak fluorescence signal and a short fluorescence lifetime due to efficient self-quenching. In the presence of enzyme hyaluronidase, the brightness and fluorescence lifetime of the sample increases with a shift in the peak emission to its original wavelength at 560 nm. The ratio of the fluorescence intensity of the HA - ADOTA probe at 560 nm and 605 nm can be used as the sensing signal for detecting hyaluronidase. Long fluorescence lifetime and red emission also makes it suitable for time-gated lifetime imaging. The proposed method makes it a rapid and sensitive assay, making it useful to assay hyaluronidase in relevant clinical samples.

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The Role of Conserved Polar Amino Acids at the Transmembrane Loop Regions of a Genetically Encoded Voltage Sensor

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To identify potential regions of the voltage-sensing domain that could shift the voltage sensitivity we aligned 183 voltage-gated sodium channels (Nav) from different organisms. Conserved polar residues were identified at multiple transmembrane loop junctions in the voltage sensing domain. Similar conservation of polar amino acids were found in the voltage sensing domain of the voltage-sensing phosphatase gene family. These conserved residues were mutated to nonpolar or oppositely charged amino acids in a Genetically Encoded Voltage Indicator (GEVI) that utilizes the voltage sensing domain of the voltage sensing phosphatase from *Ciona intestinalis*

fused to the fluorescent protein, super ecliptic pHlorin (A227D). Combined voltage clamp fuorescence measurements were made to determine their potential role in sensing voltage across the plasma membrane. Different mutations shifted the voltage sensitivity in a more positive or a more negative direction. Some of these mutations also changed the kinetics of the optical response.

Interestingly, some of these probes show a spatially dynamic internal signal from the nuclear envelope which gets brighter upon depolarization of the external membrane. These probes have a signal at the plasmamembrane which gets dimmer upon depolarizations.

Biomaterials & Biosurfaces

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A Facile Novel Method to Control Surface Topography of Conducting Polymer for Improved Coronary Stent Performance

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Endothelium lines the inner layer of all the arteries, its very important for healthy artery functions, and denudation of its layer is unavoidable during angioplasty. Thereby, drug eluting coronary stents are associated with delayed healing of the endothelial cell monolayer. Our aim was to optimise factors affecting electrochemical polymerisation and establish the influence of any achieved morphology due to optimization method in terms of re-endothelialisation of stents by assessing cell adhesion, proliferation and migration. Conducting polymer- Polypyrrole (Ppy) was used by the virtue of its application, influencing factors were identified and an optimized method was proposed for developing a consistent, reproducible method for obtaining homogeneous distinct morphologies of Ppy as stent coating. A monomer (Py) and dopant (NaSa) concentration of 0.2M and 0.1M used respectively in optimised method for 5 and 15 minutes with applied potential of 0.9V, mainly resulting in two new topographies. First named as "Coral Reef" due to its resemblance to actual coral reef, characterised by long open ended tubules ($\approx 250\mu\text{m}$), second topography also demonstrate distinct features in the form of cups and bowls, which is never reported with the aforementioned monomer and dopant using potentiostatic method. Further, a method was successfully devised to control tubules of "coral reef" topography leading to a homogeneous rough lump. SEM and AFM were used for surface characterisation. Coated wires displaying "Noble Coral Reef" morphology were seeded with ECs, which exhibited variance in adherence to Ppy coatings. 4 Bare metal stents coated with/without coral reef topography were inflated exceeding their expansion limits to examine adherence of coating and for any visible fragmentation using SEM. Despite the previously reported brittle nature of Ppy, this test successfully exhibited near perfect adherence strength. These findings of increased surface area are promising for enhanced drug delivery in drug eluting stents.

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Design of an Amyloid-Like Nanosheet with Tunable Functionality as Bio-Nanomaterials

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Amyloid protein aggregation is closely related to a variety of devastating human diseases including Alzheimer's, Huntington's and Prion disease¹. However, many functional amyloids were recently found with important physiological functions, such as long-term memory persistence, biofilm formation and hormone storage². As self-assembled protein/peptide polymers, the amyloids feature highly ordered hierarchical structure with high thermal stability and stiffness, controllable assembly, and biocompatibility. Thus, development of amyloid as functional materials becomes one of the emerging new frontiers in bio-nanomaterial field.

In our work³, we found that a heptapeptide derived from A β , the key player in Alzheimer's, is capable of forming a previously unreported giant nanosheet structure. We characterized the structure of a nanosheet by multiple biophysical approaches. Based on the structural model, we developed a series of amyloid-like nanosheets with tunable functionalities and used them as a new type of effective enhancers for retrovirus transduction. Our work demonstrates that amyloid-like nanosheets provides a robust platform for integrating different functionalities, shedding light on the development of amyloid-based bio-nanomaterials with novel functions.

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